

**CLAIMS**

We claim:

- 5        1. A method for determining the presence and/or amount of an analyte of interest  
in a test sample, said method comprising the steps of:  
    (I) applying the test sample to a test strip to form a sample mixture in a sample  
reservoir, said test strip comprising  
        (A) a chromatographic medium;  
    10      (B) the sample reservoir disposed on said chromatographic medium for  
receiving said test sample, said sample reservoir comprising  
        (i) a first detection reagent comprising  
            (a) a first detection ligand capable of selectively binding a  
                first target moiety of said analyte of interest, wherein (i) said first  
                detection ligand is conjugated with a semiconductor nanocrystal  
                which, when exposed to a light of a selected excitation wavelength,  
                is capable of emitting light of a characteristic emission peak, and  
            (ii) binding of said first detection ligand to said first target moiety  
                forms a detection complex,  
    15      (C) a capture reagent immobilized on said chromatographic medium  
within a capture region which is distinct from said sample reservoir, wherein said  
capture reagent comprises a capture ligand capable of selectively binding said first  
detection complex to form an immobilized capture complex; and  
    (C) a control ligand immobilized on said chromatographic medium within  
20      a control region distinct from said sample reservoir and said capture region,  
wherein said control ligand is capable of selectively binding said first detection  
ligand to form an immobilized control complex;  
    wherein (i) said test strip has first and second ends, said sample reservoir is  
disposed at said first end, and said capture region is interposed between said sample  
25      reservoir and said control region, (ii) said sample mixture comprises said test sample and

~~said first detection reagent, (iii) said sample mixture is transported via said chromatographic medium from said first to said second end, (iv) said first detection ligand binds said first target moiety to form said detection complex, said detection complex is bound by said capture reagent, and said first detection ligand which is not bound to said first target moiety is bound to said control ligand; and~~

~~(II) exposing said test strip to said light of a selected excitation wavelength, wherein the production of light of said characteristic emission peak in both the capture and control regions is indicative of the presence of the analyte in the test sample.~~

10 2. The method of claim 1, wherein the amount of analyte in the test sample may be quantified by measuring the quantity of light emitted by the capture region.

15 3. The method of claim 1, wherein said first detection reagent is present in said sample reservoir in a dehydrated form.

4. The method of claim 1, wherein said chromatographic medium comprises a nitrocellulose membrane.

20 5. The method of claim 1, wherein one or more of said detection reagents comprises said semiconductor nanocrystal conjugated directly to said detection ligand.

6. The method of claim 1, wherein one or more of said detection reagents comprises a microsphere conjugated directly to said detection ligand, wherein said microsphere is dyed with said semiconductor nanocrystals.

25 7. The method of claim 6, wherein said nanocrystals are disposed on the exterior surface of said microsphere.

30 8. The method of claim 6, wherein said nanocrystals are contained within the interior of said microsphere.

9. The method of claim 6, wherein said microsphere comprises a material selected from the group consisting of polystyrene, polymethylacrylate, polyacrylamide, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate and glass.

5 10. The method of claim 1, wherein said first detection ligand is a protein.

11. The method of claim 10, wherein said protein is an antibody.

12. The method of claim 10, wherein said protein is an enzyme.

10 13. The method of claim 1, wherein said first detection ligand is a nucleic acid molecule.

15 14. The method of claim 1, wherein said capture ligand is a protein.

15 15. The method of claim 14, wherein said protein is an antibody.

16. The method of claim 14, wherein said protein is an enzyme.

20 17. The method of claim 1, wherein said capture ligand is selected from the group consisting of a nucleic acid molecule, biotin and an antibody.

18. The method of claim 1, wherein said control ligand is a protein.

25 19. The method of claim 18, wherein said protein is an antibody.

20. The method of claim 18, wherein said protein is an enzyme.

30 21. The method of claim 1, wherein said control ligand is a nucleic acid molecule or biotin.

22. A method for determining the presence and/or amount of an analyte of interest in a test sample, said method comprising the steps of:

(I) applying the test sample to a test strip to form a sample mixture in a sample reservoir, said test strip comprising

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(A) a chromatographic medium;

(B) the sample reservoir disposed on said chromatographic medium for receiving said test sample, said sample reservoir comprising

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(i) a first detection reagent, comprising

(a) a first detection ligand capable of selectively binding a first target moiety of said analyte of interest,

wherein said first detection ligand is conjugated with a semiconductor nanocrystal which, when exposed to a light of a selected excitation wavelength, is capable of emitting light of a characteristic emission peak, and

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(ii) a second detection reagent comprising a second detection

ligand capable of selectively binding (a) a second target moiety of said analyte of interest, and (b) a capture ligand,

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wherein binding of said first detection ligand to said first target moiety and said second detection ligand to said second target moiety forms a detection complex,

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(C) a capture reagent immobilized on said chromatographic medium within a capture region which is distinct from said sample reservoir, wherein said capture reagent comprises a capture ligand capable of selectively binding said second detection ligand to form an immobilized capture complex; and

(D) a control ligand immobilized on said chromatographic medium within a control region distinct from said sample reservoir and said capture region, wherein said control ligand is capable of selectively binding said first detection ligand not bound to said first target moiety to form an immobilized control complex;

wherein (i) said test strip has first and second ends, said sample reservoir is disposed at said first end, and said control region is interposed between said sample reservoir and said capture region, (ii) said sample mixture comprises said test sample and said first detection reagent, (iii) said sample mixture is transported via said chromatographic medium from said first to said second end, (iv) said first detection ligand binds said first target moiety and said second detection ligand binds said second target moiety to form said detection complex, (v) said detection complex is bound by said capture reagent, and (vi) said first detection ligand which is not bound to said first target moiety is bound to said control ligand; and

10 (II) exposing said test strip to said light of a selected excitation wavelength, wherein the production of light of said characteristic emission peak in both the capture and control regions is indicative of the presence of the analyte in the test sample.

15 23. The method of claim 22, wherein the amount of analyte in the test sample may be quantified by measuring the quantity of light emitted by the capture region.

20 24. The method of claim 22, wherein said first detection reagents are present in said sample reservoir in a dehydrated form.

25 25. The method of claim 22, wherein said chromatographic medium comprises a nitrocellulose membrane.

26. The method of claim 22, wherein one or more of said detection reagents comprises said semiconductor nanocrystal conjugated directly to said detection ligand.

27. The method of claim 22, wherein one or more of said detection reagents comprises a microsphere conjugated directly to said detection ligand, wherein said microsphere is dyed with said semiconductor nanocrystals.

28. The method of claim 27, wherein said nanocrystals are disposed on the exterior surface of said microsphere.

29. The method of claim 27, wherein said nanocrystals are contained within the 5 interior of said microsphere.

30. The method of claim 27, wherein said microsphere comprises a material selected from the group consisting of polystyrene, polymethylacrylate, polyacrylamide, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate and glass.

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31. The method of claim 22, wherein said first detection ligand is a protein.

32. The method of claim 31, wherein said protein is an antibody.

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33. The method of claim 31, wherein said protein is an enzyme.

34. The method of claim 22, wherein said first detection ligand is nucleic acid molecule.

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35. The test strip of claim 22, wherein said capture ligand is a protein.

36. The method of claim 35, wherein said protein is an antibody.

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37. The method of claim 35, wherein said protein is an enzyme.

38. The method of claim 22, wherein said capture ligand is selected from the group consisting of a nucleic acid molecule, biotin and an antibody.

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39. The method of claim 22, wherein said control ligand is a protein.

40. The method of claim 39, wherein said protein is an antibody.
41. The method of claim 39, wherein said protein is an enzyme.
- 5 42. The method of claim 22, wherein said control ligand is a nucleic acid molecule or biotin.
43. The method of claim 22, wherein said second detection ligand is a protein.
- 10 44. The method of claim 39, wherein said protein is an antibody.
45. The method of claim 39, wherein said protein is an enzyme.
- 15 46. The method of claim 22, wherein said second detection ligand is a nucleic acid molecule.
47. The method of claim 22, wherein said second detection ligand is a bioavidylated antibody and said capture ligand is streptavidin.
- 20 48. The method of claim 22, wherein said second detection ligand is a bioavidylated antibody and said capture ligand is avidin.
49. The method of claim 22, wherein said second detection ligand is an antibody conjugated to digoxigenin and said capture ligand is anti-digoxigenin.
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50. A method for determining the presence and/or amount of an analyte of interest in a test sample, said method comprising the steps of:
- (I) applying the test sample to a test strip to form a sample mixture in a sample reservoir, said test strip comprising
- 30 (A) a chromatographic medium;

- (B) the sample reservoir disposed on said chromatographic medium for receiving said test sample, said sample reservoir comprising
- (i) a first detection reagent comprising
- (a) a first detection ligand capable of selectively binding a first target moiety of said analyte of interest, wherein (i) said first detection ligand is conjugated with a semiconductor nanocrystal which, when exposed to a light of a selected excitation wavelength, is capable of emitting light of a characteristic emission peak, and
- (ii) binding of said first detection ligand to said first target moiety forms a first detection complex,
- (C) a capture reagent immobilized on said chromatographic medium within a capture region which is distinct from said sample reservoir, wherein said capture reagent comprises a capture ligand capable of selectively binding said first detection complex to form an immobilized capture complex; and
- (D) a control reagent immobilized on said chromatographic medium within a control region distinct from said sample reservoir and said capture region, wherein said control ligand is capable of selectively binding said first detection ligand to form an immobilized control complex;
- wherein (i) said test strip has first and second ends, said sample reservoir is disposed at said first end, and said capture region is interposed between said sample reservoir and said control region, (ii) said sample mixture comprises said test sample and said first detection reagent, (iii) said sample mixture is transported via said chromatographic medium from said first to said second end, (iv) said first detection ligand binds said first target moiety and said second detection ligand binds said second target moiety to form said detection complex, (v) said detection complex is bound by said capture reagent, and (vi) said first detection ligand which is not bound to said first target moiety is bound to said control ligand; and
- (II) exposing said test strip to said light of a selected excitation wavelength, wherein the production of light of said characteristic emission peak in both the capture and control regions is indicative of the presence of the analyte in the test sample.

51. The method of claim 50, wherein the amount of analyte in the test sample may be quantified by measuring the quantity of light emitted by the capture region.

5 52. The method of claim 50, wherein said first detection reagents are present in said sample reservoir in a dehydrated form.

53. The method of claim 50, wherein said chromatographic medium comprises a nitrocellulose membrane.

10 54. The method of claim 50, wherein one or more of said detection reagents comprises said semiconductor nanocrystal conjugated directly to said detection ligand.

15 55. The method of claim 50, wherein one or more of said detection reagents comprises a microsphere conjugated directly to said detection ligand, wherein said microsphere is dyed with said semiconductor nanocrystals.

56. The method of claim 55, wherein said nanocrystals are disposed on the exterior surface of said microsphere.

20 57. The method of claim 55, wherein said nanocrystals are contained within the interior of said microsphere.

25 58. The method of claim 55, wherein said microsphere comprises a material selected from the group consisting of polystyrene, polymethylacrylate, polyacrylamide, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate and glass.

59. The method of claim 50, wherein said first detection ligand is a protein.

60. The method of claim 59, wherein said protein is an antibody.

61. The method of claim 59, wherein said protein is an enzyme.
62. The method of claim 50, wherein said first detection ligand is a nucleic acid molecule.
- 5 63. The method of claim 50, wherein said capture ligand is a protein.
64. The method of claim 63, wherein said protein is an antibody.
- 10 65. The method of claim 63, wherein said protein is an enzyme.
66. The method of claim 50, wherein said capture ligand is selected from the group consisting of a nucleic acid molecule, biotin and an antibody.
- 15 67. The method of claim 50, wherein said control ligand is a protein.
68. The method of claim 67, wherein said protein is an antibody.
69. The method of claim 67, wherein said protein is an enzyme.
- 20 70. The method of claim 50, wherein said control ligand is a nucleic acid molecule or biotin.
71. A test strip for determining the presence and/or amount of an analyte of interest suspected of being present in a test sample, comprising:  
(I) a chromatographic medium;  
(II) a sample reservoir disposed on said chromatographic medium for receiving said test sample, said sample reservoir comprising  
(A) a first detection reagent comprising

- (i) a first detection ligand capable of selectively binding a first target moiety of said analyte of interest, wherein (a) said first detection ligand is conjugated with a semiconductor nanocrystal which, when exposed to a light of a selected excitation wavelength, is capable of emitting light of a characteristic emission peak, and (b) binding of said first detection ligand to said first target moiety forms a first detection complex,
- (II) a capture reagent immobilized on said chromatographic medium within a capture region which is distinct from said sample reservoir, wherein said capture reagent comprises a capture ligand capable of selectively binding said first detection complex to form an immobilized capture complex; and
- (III) a control ligand immobilized on said chromatographic medium within a control region distinct from said sample reservoir and said capture region, wherein said control ligand is capable of selectively binding said first detection ligand to form an immobilized control complex.

72. The test strip of claim 71, wherein said first detection reagent is present in said sample reservoir in a dehydrated form.

73. The test strip of claim 71, wherein said chromatographic medium comprises a nitrocellulose membrane.

74. The test strip of claim 71, wherein one or more of said detection reagents comprises said semiconductor nanocrystal conjugated directly to said detection ligand.

75. The test strip of claim 71, wherein one or more of said detection reagents comprises a microsphere conjugated directly to said detection ligand, wherein said microsphere is dyed with said semiconductor nanocrystals.

76. The test strip of claim 75, wherein said nanocrystals are disposed on the exterior surface of said microsphere.

5 77. The test strip of claim 75, wherein said nanocrystals are contained within the interior of said microsphere.

78. The test strip of claim 75, wherein said microsphere comprises a material selected from the group consisting of polystyrene, polymethylacrylate, polyacrylamide, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate and glass.

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79. The test strip of claim 71, wherein said first detection ligand is a protein.

80. The test strip of claim 79, wherein said protein is an antibody.

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81. The test strip of claim 79, wherein said protein is an enzyme.

82. The test strip of claim 71, wherein said first detection ligand is a nucleic acid molecule.

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83. The test strip of claim 71, wherein said capture ligand is a protein.

84. The test strip of claim 83, wherein said protein is an antibody.

85. The test strip of claim 83, wherein said protein is an enzyme.

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86. The test strip of claim 71, wherein said capture ligand is selected from the group consisting of a nucleic acid molecule, biotin and an antibody.

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87. The test strip of claim 71, wherein said control ligand is a protein.

88. The test strip of claim 87 wherein said protein is an antibody.
89. The test strip of claim 87, wherein said protein is an enzyme.
- 5 90. The test strip of claim 71, wherein said control ligand is a nucleic acid molecule or biotin.
91. The test strip of claim 71, wherein said test strip has first and second ends, said sample reservoir is disposed at said first end, and said capture region is interposed between said sample reservoir and said control region, and wherein further said capture ligand is capable of binding a second target moiety of the analyte, and said control ligand is capable of binding said first detection ligand which is not bound to said first target moiety.
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- 15 92. The test strip of claim 71, wherein said test strip has first and second ends, said sample reservoir is disposed at said first end, and said control region is interposed between said sample reservoir and said capture region, and wherein said control ligand is capable of binding said first detection ligand which is not bound to said first target moiety, and said capture ligand is capable of binding said first detection complex.
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93. The test strip of claim 92, wherein said control ligand comprises said analyte of interest.
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94. The test strip of claim 92, wherein said control ligand comprises said first target moiety of said analyte of interest.
95. A test strip for determining the presence and/or amount of an analyte of interest suspected of being present in a test sample, comprising:  
(I) a chromatographic medium;

- (II) a sample reservoir disposed on said chromatographic medium for receiving  
said test sample, said sample reservoir comprising  
(A) a first detection reagent comprising  
(i) a first detection ligand capable of selectively binding a first  
target moiety of said analyte of interest, wherein said first detection ligand  
is conjugated with a semiconductor nanocrystal which, when exposed to a  
light of a selected excitation wavelength, is capable of emitting light of a  
characteristic emission peak,  
(B) a second detection reagent comprising  
(i) a second detection ligand capable of selectively binding (a) a  
second target moiety of said analyte of interest, and (b) a capture ligand,  
(III) a capture reagent immobilized on said chromatographic medium within a  
capture region which is distinct from said sample reservoir, wherein said capture reagent  
comprises a capture ligand capable of selectively binding said second detection ligand to  
form an immobilized capture complex; and  
(IV) a control ligand immobilized on said chromatographic medium within a  
control region distinct from said sample reservoir and said capture region, wherein said  
control ligand is capable of selectively binding said first detection ligand not bound to  
said first target moiety to form an immobilized control complex, wherein said test strip  
has first and second ends, said sample reservoir is disposed at said first end, and said  
capture region is interposed between said sample reservoir and said control region.

96. The test strip of claim 95, wherein said second detection ligand is a protein.  
25 97. The test strip of claim 96, wherein said protein is an antibody.  
98. The test strip of claim 96, wherein said protein is an enzyme.  
30 99. The test strip of claim 95, wherein said second detection ligand is a nucleic  
acid molecule.

100. The test strip of claim 95, wherein said second detection ligand is a bioatinylated antibody and said capture ligand is streptavidin.

101. The test strip of claim 95, wherein said second detection ligand is a bioatinylated antibody and said capture ligand is avidin.

102. The test strip of claim 95, wherein said second detection ligand is an antibody conjugated to digoxigenin and said capture ligand is anti-digoxigenin.